Mutant-specific oligonucleotide primers used for mutant number 1. Mutated nucleotide underlined

Ī	LO	20	30	40	50	
	-	_	-	-	-	
Bet v 1 sense	5'-	AATTATGAGAC	TGAGACCAC	CTCTGTTATC	CCAGCAGCTCG	; -3'
Bet v 1 non-sense	3'-	TTAATACTCTC	actetegie	gagacaatag	GGTCGTCGAGO	: -5'
sense primer	5 '-		TGAGACCCC	CTCTGTTATC	CCAG	-3'
hon-sense primer	31-	ATACTCTG	ACTCTEGGG	GAGACA		_61

20

10

Oligonucleotide primers for site directed mutagenesis of Bet v 1 (No. 2801).

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all
               1: 163Bv, 15-mer 5'-GTTGCCAACGATCAG
     sense
               2: 184Bv, 23-mer 5'-TGAGACCCCCTCTGTTATCCCAG
     sense
1
     non-sense 3: 185Bv, 23-mer 5'-ACAGAGGGGGTCTCAGTCTCATA
2
     sense
               4: 1868v, 31-mer 5'-GATACCCTCTTTCCACAGGTTGCACCCCAAG
2
    non-sense 5: 1878v, 31-mer 5'-ACCTGTGGAAAGAGGGTATCGCCATCAAGGA
               6: 188Bv, 23-mer 5'-AACATTTCAGGAAATGGAGGGCC
3
     non-sense 7: 1898v, 23-mer 5'-TTTCCTGAAATGTTTTCAACACT
               8: 190Bv, 23-mer 5'-TTAAGAACATCAGCTTTCCCGAA
     sense
4
     non-sense 9: 1918v, 23-mer 5'-AGCTGATGTTCTTAATGGTTCCA
5
              10: 192Bv, 23-mer 5'-GGACCATGCAAACTTCAAATACA
5
    non-sense 11: 1938v, 23-mer 5'-AGTTTGCATGGTCCACCTCATCA
6
              12: 194Bv, 23-mer 5'-TTTCCCTCAGGCCTCCCTTTCAA
б
    non-sense 13: 195Bv, 23-mer 5'-AGGCCTGAGGGAAAGCTGATGTT
7
              14: 196Bv, 24-mer 5'-TGAAGGATCTGGAGGGCCTGGAAC
    sense
7
    non-sense 15: 1978v, 24-mer 5'-CCCTCCAGATCCTTCAATGTTTTC
              16: 1988v, 24-mer 5'-GGCAACTGGTGATGGAGGATCCAT
    non-sense 17: 1998v, 24-mer 5'-CCATCACCAGTTGCCACTATCTTT
8
all non-sense 18: 200Bv, 15-mer 5'-CATGCCATCCGTAAG
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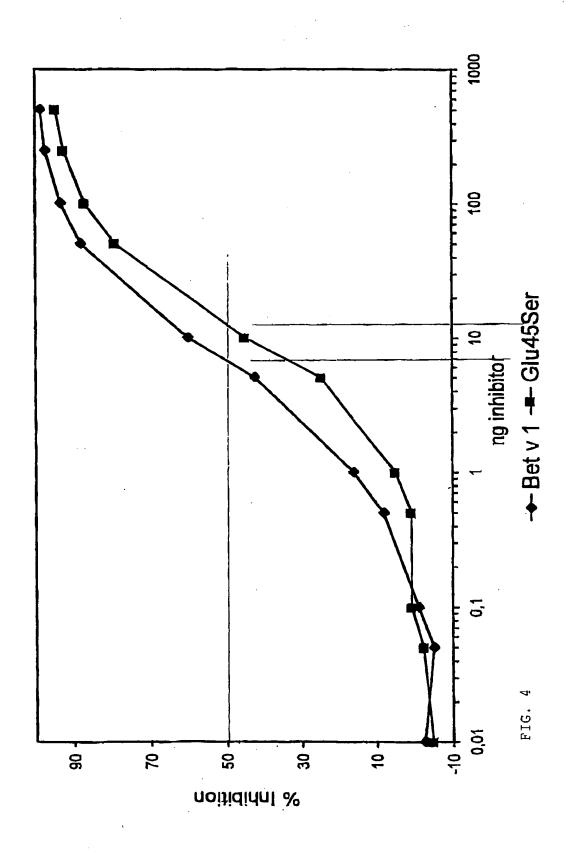
Overview of all mutations

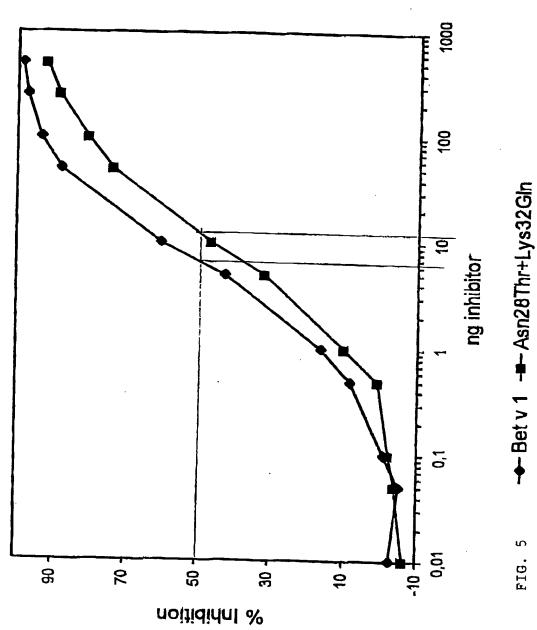
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				rg <u>a</u> t														AG	Cagt	120
A	r	Ι	L	D-G	G	Đ	N-T	L	F	P	K-Q	٧	A	P	Q	A	I	S	s	40
3 (GA-TC) 7 (AA-TC) 4 (G-C) 6 (GA-TC)																				
GTT	GAA	AAC	AT?	[GAA	GGA	<u> AA'</u>	GGA	GGG	CCT	5G,F	ACC/	\TT	A AG	AAG	ATC	AGC	TTT	CCC	<u>GA</u> A	180
v	E	N	I	E-S	G	N-5	G	G	P	G	T	I	K	K-N	I	\$	F	Þ	E-5	60
	5 (CA-TG)																			
GGC	CTC	CCT	TTC	'AAG	rac	GTC	AAG	GΑÇ	'AGAC	i'T'	GATO	AG	GT G	GAC	CAC	<u>A</u> CA	AAC	TTC	AAA	240
G	L	P·	F	ĸ	Y	v	ĸ	D	R	v	D	E	٧	D	H	T-A	N	f	K	80
TAC	ጓ ዲቲ"	ľac.	AGC	GTG	NTC	GAG	GGC	GGI	,CCC;	ďΣ	NGGC0	ac.	LCA	TTG	SAG	AAG	ATC	TCC	:AAC	300
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														. –	_			_	••	100
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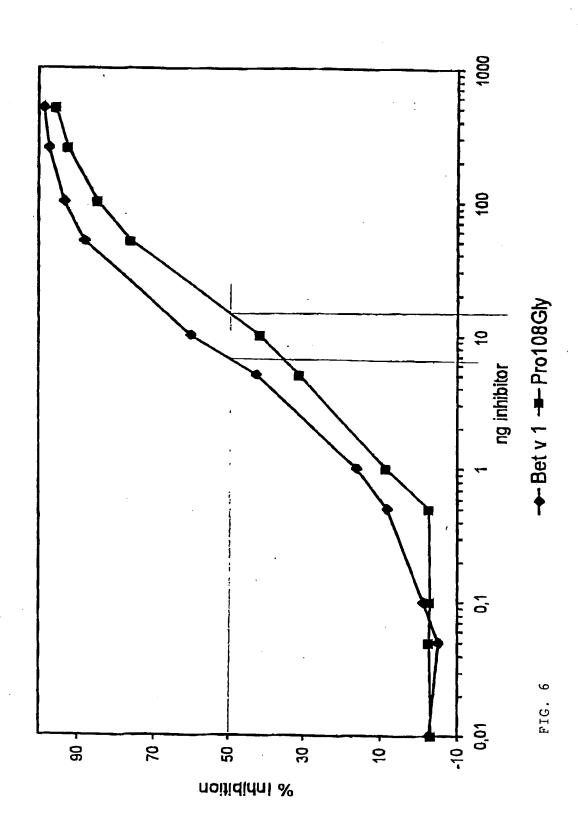
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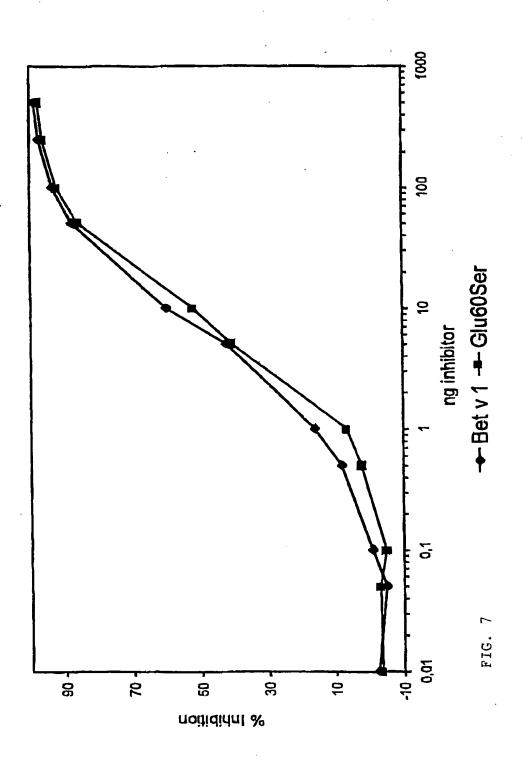
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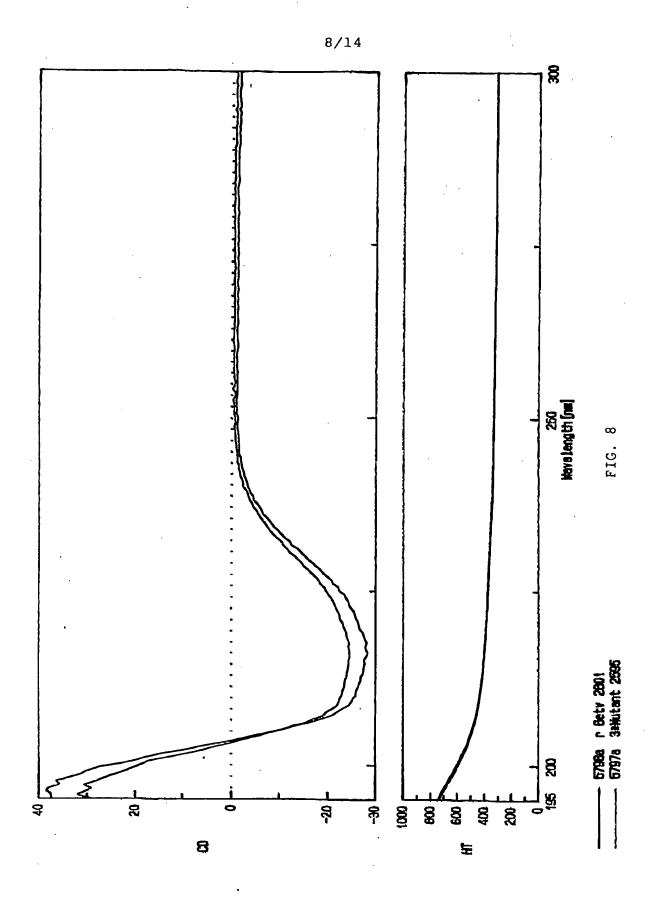
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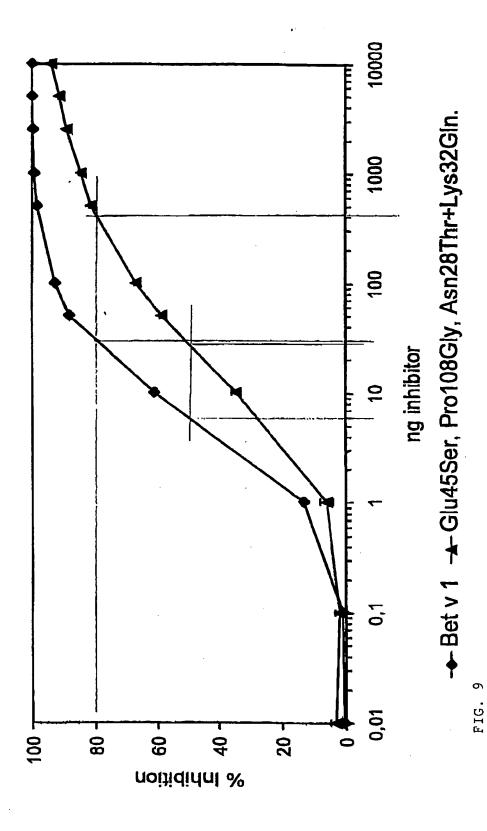












Conserved residues among Vespula antigen 5

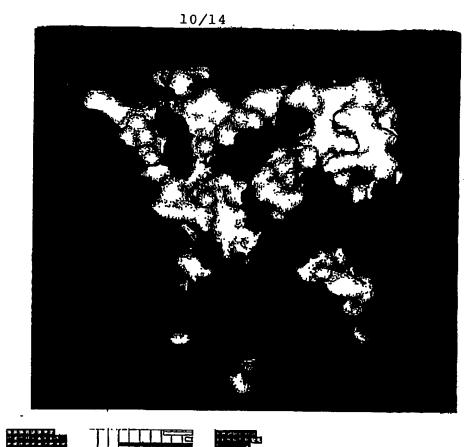




FIG. 10



Mutant-specific oligonucleotide primers used for Ves v 5 mutants. Mutated nucleotides underlined.

Ves v 5 mutant 1 (K72A)

Ves v 5 sense Ves v 5 non-sense sense primer non-sense primer		
Ves v 5 mutant 2 (Y9		_
Ves v 5 non-sense	5'- GGCTAATCAATGTCAATATGGTCACGATACTTGCAGGGATG -:	
	3 - CCGATTAGTTACAGTTATACCAGTGCTATGAACGTCCCTAC -	5 ~
sense primer	•	3 -
non-sense primer	3 - TTAGTTACAGTT <u>CG</u> ACCAGTG	5 -



Oligonucleotide primers for site directed mutagenesis of Ves v 5.

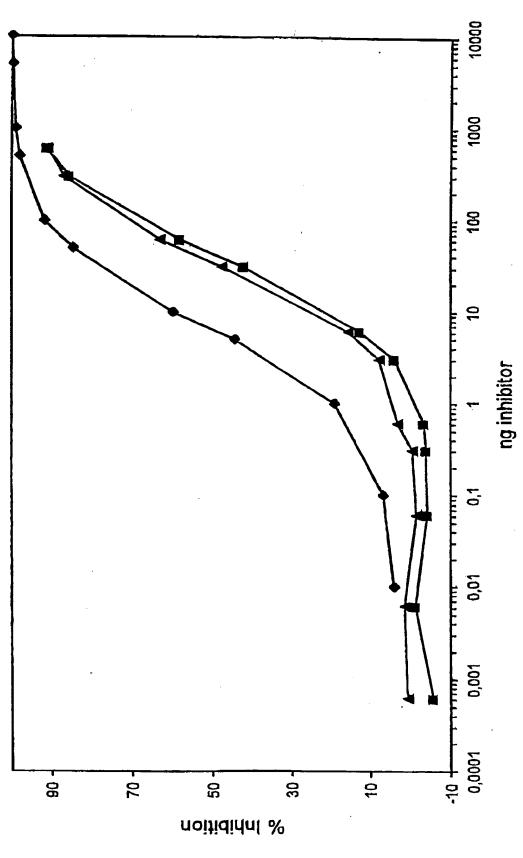
all sense 1: XhoI start, 38-mer:

Ecori

1	sense non-sense		K72AB K72Aa		mer mer	5´-caagcggctaatatgaaaaat 5´-catattagccgctggaggctg
2 2	nou-serse Tou-serse		Y96As Y96Aa		mer mer	5 ~- TGTCAAGCTGGTCACGATACT 5 ~- GTGACCAGCTTGACATTGATT
all	non-sense	7:	CT-pPICZ	αA,	21-mer	5 - ATTCATCAGCTGCGAGATAGG

Overview of Ves v 5 mutations

AA	AA:	I'TA'	TTO	TAA	AAT.	AAA	ATG	Thirth	GAD.	שממ	A cic	ተረተ	CCN	m	~~					
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Y	Q	T	K	st	qo															204
	AGT S AAM K ACT TGC GCT A AAT V CAC H TATO	AGTOTT S L AAACAM K Q ACTAGM T R GACGAG D E TGCAGG C R GATAAA A K AATCCT N P GTTTGG V W CACAAA H K	AGTCTTANA S L K AAACAAGAC K Q D ACTAGAGGT T R G GACGAGTT D E L TGCAGGGAT C R D GCTAAATAC A K Y AATCCTAAG N P K GTTTGGGCT V W A CACAAACAT H K H TATCAAACA	AGTCTTARACCO S L K P ARACAAGACATO K Q D I ACTAGAGGTAAT T R G N GACGAGTTAGCT D E L A TGCAGGGATGTA C R D V GCTARATACGAT A K Y D AATCCTAAGAAA N P K K GTTTGGGCTAAC V W A N CACAAACATTAC H K H Y TATCAAACAAG	AGTCTTARACCGAR' S L K P N ARACAAGACATCTTT K Q D I L ACTAGAGGTAATCCT T R G N P GACGAGTTAGCTTAT D E L A Y TGCAGGGATTAGCTTAT C R D V A GCTARATACGATGAT A K Y D D AATCCTAAGAARAAG N P K K K GTTTGGGCTAACACC V W A N T CACAAACATTACCTT H K H Y L TATCAAACAAAGTAA	AGTOTTARACCGAATTGG S L K P N C ARACAAGACATCTTARACC K Q D I L K ACTAGAGGTARTCCTGGE T R G N P G GACGAGTTAGCTTATGTC D E L A Y V TGCAGGGATGTAGCAAAA C R D V A K GCTARATACGATGATCCA A K Y D D P AATCCTAAGAAAAAGTTT N P K K K F GTTTGGGCTAACACCAAG V W A N T K CACAAACATTACCTTGTA	AGTCTTAAACCGAATTGCGG S L K P N C G AAACAAGACATCTTAAAGGAC K Q D I L K E ACTAGAGGTAATCCTGGACCE T R G N P G P GACGAGTTAGCTTATGTCGCC D E L A Y V A TGCAGGGATGTAGCAAAATAT C R D V A K Y GCTAAATACGATGATCCAGTT A K Y D D P V AATCCTAAGAAAAAAGTTTTCG N P K K K F S GTTTGGGCTAACACCAAGGAA V W A N T K E CACAAACATTACCTTGTATGT H K H Y L V C	AGTCTTARACCGARTTGCGTAR S L K P N C G N ARACAAGACATCTTARAGGAGCAC K Q D I L K E H ACTAGAGGTARTCCTGGACCACAC T R G N P G P Q GACGAGTTAGCTTATGTCGCCCAR D E L A Y V A Q TGCAGGGATTAGCTTAGCAAAATATCAC C R D V A K Y Q GCTARATACGATGATCCAGTTAAR A K Y D D P V K AATCCTAAGARARAGTTTTCGGGA N P K K K F E G GTTTGGGCTAACACCAAGGAAGTT V W A N T K E V CACAAACATTACCTTGTATGTAAT H K H Y L V C N	AGTCTTARACCGAATTGCGGTAATAAG S L K P N C G N K AAACAAGACATCTTAAAGGAGCACAAT K Q D I L K E H N ACTAGAGGTAATCCTGGACCACAGCCT T R G N P G P Q P GACGAGTTAGCTTATGTCGCCCAAGTG D E L A Y V A Q V TGCAGGGATGTAGCAAAATATCAGGTT C R D V A K Y Q V GCTAAATACGATGATCCAGTTAAACTA A K Y D D P V K L AATCCTAAGAAAAAGTTTTCGGGAAAC N P K K K F E G N GTTTGGGCTAACACCAAGGAAGTTGGT V W A N T K E V G CACAAACATTACCTTGTATGTAATTAT H K H Y L V C N Y TATCAAACAAAGTAA	AGTCTTAAACCGAATTGCGGTAATAAGGTA S L K P N C G N K V AAACAAGACATCTTAAAAGGAGCACAATGAC K Q D I L K E H N D ACTAGAGGTAATCCTGGACCACAGCCTCCA T R G N P G P Q P P GACGAGTTAGCTTATGTCGCCCAAGTGTGGA C R D V A K Y Q V G GCTAAATACGATGATCCAGGTTAAACTAGTT A K Y D D P V K L V AATCCTAAGAAAAAAGTTTTCGGGAAACGAC N P K K K F E G N D GTTTGGGCTAACACCAAGGAAGTTGGTTGT V W A N T K E V G C CACAAACATTACCTTGTATGTAATTATGGAA H K H Y L V C N Y G	AGTCTTARACCGAATTGCGGTAATAAGGTAGTTGS L K P N C G N K V V AAACAAGACATCTTAAAGGAGCACAATGACTTK Q D I L K E H N D F ACTAGAGGTAATCCTGGACCACAGCCTCCAGCCTCAGCCTTR G N P G P Q P P A GACGAGTTAGCTTATGTCGCCCAAGTGTGGGCTD E L A Y V A Q V W A TGCAGGGATGTAGCAAAATATCAGGTTGGACAACCTTAAAACCAAACAAA	AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGT S L K P N C G N K V V V AAACAAGACATCTTAAAAGGAGCACAATGACTTTAGG K Q D I L K E H N D F R ACTAGAGGTAATCCTGGACCACAGCCTCCAGCGAAG T R G N P G P Q P P A R GACGAGTTAGCTTATGTCGCCCAAGTGTGGGCTAAT D E L A Y V A Q V W A N TGCAGGGATGTAGCAAAATATCAGGTTGGACAAAAC C R D V A K Y Q V G Q N GCTAAATACGATGATCAGTTAAACTAGTTAAAATG A K Y D D P V K L V K M AATCCTAAGAAAAAAGTTTTCGGGAAACGACTTTCTG N P K K K F S G N D F L GTTTGGGCTAACACCAAGGAAGTTGGTTGTGGAAGT V W A N T K E V G C G S CACAAACATTACCTTGTATGTAATTATGGACCCAGC H K H Y L V C N Y G P S	AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATC S L K P N C G N K V V V S AAACAAGACATCTTAAAGGAGCACAATGACTTTAGACAA K Q D I L K E H N D P R Q ACTAGAGGTAATCCTGGACCACAGCCTCCAGCGAAGAAT T R G N P G P Q P P A K N GACGAGTTAGCTTATGTCGCCCAAGTGTGGGCTAATCAA D E L A Y V A Q V W A N Q TGCAGGGATGTAGCAAAAATATCAGGTTGGACAAAACGTP C R D V A K Y Q V G Q N V GCTAAATACGATGATCCAGTTAAACTAGTTAAAATGTGC A K Y D D P V K L V K M W AATCCTAAGAAAAAGTTTTCGGGAAACGACTTTCTGAAA N P K K K F S G N D F L K GTTTGGGCTAACACCAAGGAAGTTGGTTGTGGAAGTATA V W A N T K E V G C G S I CACAAACATTACCTTGTATGTAATTATGGACCCAGCGGAA H K H Y L V C N Y G P S G	AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCCTAGGTAACCGAATTGCGGTAATAAGGTAGTGGTATCCTAGGTAACAACAACCTTTAAAAGGAGAGACAATGACTTTAGACAAAAACAAAACGACCTCCAGGCGAAGGAACAATAACTTAGACAAAAACGAAGACTTAAAAACGTAGGCAAGAGATTAACCAAAAAACGAAGACTTAGAAAAACGTAGCCCCRAGGTGTGGAACAAAAACGTAGCCCCRAGGTGTGGAAAAAACGTAGCCCCCRAGGTGTGGAAAAAACGTAGCCCCCRAGTGTGGAAAAAACGTAGCCCCCRAGTGTGGAAAAAACGTAGCCCCCRAGTGTGGAAAAAACGTAGCCCCCRAGTGTGGAAAAAACGTAGCCCCCRAGTGTAGAAAACGTAGCCCCCRAGTGTAGAAAACGTAGCCCCCCCCAAGTGTGGAAAAACGTAGCCCCCCCC	AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCCTATGGT S L K P N C G N K V V V S Y G AAACAAGACATCTTAAAGGAGCACAATGACTTTAGACAAAAAATT K Q D I L K E H N D F R Q K I L[172A] (AAG ACTAGAGGTAATCCTGGACCAAGGCTCCAGCGAAGAATATATAAA T R G N P G P Q P P A K N M K GACGAGTTAGGTTATGTCGCCCAAGTGTGGGCTAATCAATGTCAA D E L A Y V A Q V W A N Q C Q TGCAGGGATGTAGCAAAAATATCAGGTTGGACAAAACGTAGCCTTA C R D V A K Y Q V G Q N V A L GCTAAATACGATGATCCAGGTTAAACTAGTTAAAATGTGGGAAGAT A K Y D D P V K L V K M W E D AATCCTAAGAAAAAGTTTCGGGAAACGACTTTCTGAAAACCGGC N P K K K F S G N D F L K T G GTTTGGGCTAACACCAAGGAAGTTGGTTGTGGAAGTATAAAATAC V W A N T K E V G C G S I K Y CACAAACATTACCTTGTATGTAATTATGGACCCAGCGGAAACTTTT H K H Y L V C N Y G P S G N F	AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCCTATGGTCT. S L K P N C G N K V V V S Y G L AAACAAGACATCTTAAAGGAGCACAATGACTTTAGACAAAAAAAA	AGTCTTARACCGAATTGCGGTAATAAGGTAGTGTATCCTATGGTCTAACCG S L K P N C G N K V V V S Y G L T AAACAAGACATCTTAAAGGAGCACAATGACTTTAGACAAAAAATTGCACGG K Q D I L K E H N D F R Q K I A R L[K72A] (AAG-GCT) ACTAGAGGTAATCCTGGACCACAGCCTCCAGCGAAGAATATGAAAAATTTCAATTTCAATGAAGAATATGAAAAATTTTCAATATGAACAAAAAAATTTCAATATGAAAAAAAA	AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCCTATGGTCTAACGAAA S L X P N C G N K V V V S Y G L T K AAACAAGACATCTTAAAAGGAGCACAATGACTTAGACAAAAAAATTGCACGAGGG K Q D I L K E H N D F R Q K I A R G ACTAGAGGTAATCCTGGACCACAGCCTCCAGCGAAGAATATGAAAAATTTGCACGAGGG T R G N P G P Q P P A K N M K N L V 2[Y96A] GACGAGTTAGCTTATGTCGCCCAAGTGTGGGCTAATCAATGTCAATATTGGTCAC D E L A Y V A Q V W A N Q C Q Y G H TGCAGGGATGTAGCAAAATATCAGGTTGGACAAAACGTAGCCTTAACAGGTAGC C R D V A K Y Q V G Q N V A L T G S GCTAAATACGATGATCCAGTTAAACTAGTTAAAATGTGGGAAGATGAACTGAAA A K Y D D P V K L V K M W E D E V K AATCCTAAGAAAAAACTTTCGGGAAAACGACTTTCTGAAAACCGGCCATTACACT N P K K K F S G N D F L K T G H Y T GTTTGGGCTAACACCAAGGAAGTTGGTTGTGGAAGTTAAAATACATTCAAGAGG V W A N T K E V G C G S I K Y I Q E CACAAACATTACCTTGTATGTAATTATGGACCCAGCGGAAACTTTAAGAATGAGG H K H Y L V C N Y G P S G N F K N E	AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCCTATGGTCTAACGAAACAI S L K P N C G N K V V V B Y G L T K Q AAACAAGACATCTTAAAGGAGCACAATGACTTTAGACAAAAAATTGGACGAGGATTG K Q D I L K E H N D F R Q K I A R G L L[K72A] (AAG-GCT) ACTAGAGGTAATCCTGGACCACAGCCTCCAGCGAAGAATATTTGGTATGGTATGGTATAGAGAGAG	T R G N P G P Q P P A R N M K N L V W N GACGAGTTAGCTTATGTCGCCCAAGTGTGGGCTTCAATGTCAATGTCAATAGTTAGGTACCTCAAGTGTGGGCTTAGCAATATGTCAACAGGTAGCACGGCT D E L A Y V A Q V W A N Q C Q Y G H D T TGCAGGGATTAGCTTAGCTAAAATATCAGGTTGGAACAAAACGTAGCCTTAACAGGTAGCACGGCT C R D V A K Y Q V G Q N V A L T G S T A GCTAAATACGATGATCCAGTTAAACTAGTTAAAATGTGGGAAGATCAAGTGAAAGATTAT A K Y D D P V K L V K M W E D E V K D Y AATCCTAAGAAAAAGTTTTCGGGAAACGACTTTCTGAAAACCGGCCATTACACTCAAATG N P K K K F S G N D F L K T G H Y T Q M GTTTGGGCTAACACCAAGGAAGTTGGTTGTGGAAGTATAAAATACATTCAAGAGAAAATGC V W A N T K E V G C G S I K Y I Q E K W CACAAACATTACCTTGTATGTAATTATGGACCCAGCGGAAACTTTAAGAATGAGGAACTT H K H Y L V C N Y G P S G N F K N E E L TATCAAACAAAGTAA



-+- r Ves v 5 -=- Lys72Aia -≠- Tyr96Aia